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Edgewood Arsenal, Aberdeen Proving Ground, Md

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A review and evaluation of available processes for detoxification of the first group of hazardous materials was conducted during Phase I of the interagency agreement (1974 calendar year). The processes used for specific hazardous materials were identified. One process found during this literature survey described the reaction of PCB's with amines to give a variety of products that were supposed to have useful properties. Two of these products that were available were obtained and evaluated toxicologically. Both proved to be higly toxic.

Products produced by catalytic decomposition were also evaluated for toxicity. In many cases the products were not adequately identified chemically and accurate toxicological evaluation could not be accomplished.

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ASSESSMENT OF TECHNIQUES FOR DETOXIFICATION OF SELECTED HAZARDOUS MATERIALS

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FOREWORD

The Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our natural environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems for the prevention, treatment, and management of wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, for the preservation and treatment of public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research; a most vital communications link between the researcher and the user community.

This report discusses some of the processes used to detoxify pesticides and gives test results for products produced by these processes.

Francis T. Mayo, Director
Municipal Environmental Research
Laboratory

PREFACE

The Environmental Protection Agency has identified a series of hazardous and toxic materials which were manufactured on a large scale during the past few years and now have been found to be harmful. These are characterized by the polychlorinated hydrocarbon insecticides, polychlorinated biphenyls (PCB's) and benzidine. In addition to these materials the EPA has listed 45 chemical pesticides that, because of their widespread distribution, present difficult disposal problems. The first group of materials, which includes four of the pesticides, is no longer to be used and is scheduled for detoxification. Proper disposal of all of these hazardous materials pose problems that are very serious from a health hazard viewpoint and can be very difficult to solve. Municipal waste disposal plants are generally not capable of treating such products, and their release into streams or improperly designed land disposal sites has led to widespread and highly undesirable environmental contamination.

Studies of decontamination methods have been too limited to provide a valid basis for the selection of a suitable detoxification procedure. Studies that have been made have rarely included an adequate assessment of the toxicity of the products obtained. No matter how free of toxicity, the materials produced should be biodegradable and not accumulate in the food chain. In general, the presence of chlorine atoms in chemical compounds contributes to resistance to biodegradation and poor water solubility coupled with high oil solubility. This often leads to accumulation in the food chain and deposition in the body fats.

One stipulation of this agreement was that products produced by Edgewood Arsenal and other EPA Contractors would be subjected to toxicological evaluation. Edgewood Arsenal has been engaged in the study of methods for the detoxification of toxic materials for many years. Continuing studies of this type are a fundamental part of Edgewood Arsenal's basic mission program. This report describes some of the techniques for detoxification and gives results of toxicological studies conducted on compounds produced by these techniques.

ABSTRACT

A review and evaluation of available processes for detoxification of the first group of hazardous materials was conducted during Phase I of the interagency agreement (1974 calendar year). The processes used for specific hazardous materials were identified. One process found during this literature survey described the reaction of PCB's with amines giving a variety of products that were supposed to have useful properties. Two of these products that were available were obtained and evaluated toxicologically. Both proved to be highly toxic.

Products produced by catalytic decomposition at Worcester Polytechnic Institute were to be studied for toxicity; however, some samples were not large enough for adequate testing and some were insoluble. In many cases the products were not adequately identified as to composition and concentration. For these reasons, though some of the products may have been reduced in toxicity, accurate toxicological evaluation could not be accomplished.

Using their expertise in detoxification methods, personnel from the Chemical Laboratory at Edgewood Arsenal have developed a process by which a number of the chlorinated hydrocarbon pesticides have been converted into water soluble compounds. Acute toxicological studies indicated that these products may be less toxic than the pesticides from which they were made. As they are considerably more water soluble than the parent insecticide it was hoped that they would not accumulate in wildlife or man. This should be beneficial for purposes of health and the environment. Unfortunately bioaccumulation studies have not been performed to prove this point. In the preliminary studies biodegradation did not occur.

Incinerator residue samples, produced by Midwest Research Institute, were insoluble in all commonly used solvents so intravenous mammalian evaluations could not be run. Some of the residues were pulverized and put into fish tanks and screened for 96-hr toxicity to bluegills. All but one of the samples were nontoxic at concentrations up to 1000 mg/l. The one exception was considered practically nontoxic with a 96-hr TL50 of 320 mg/l.

This report was submitted in fulfillment of Interagency Agreements EPA-IAG-D4-0429, EPA-IAG-D5-0429 and EPA-IAG-D6-0429 by the Toxicology Division, Chemical Laboratory, Edgewood Arsenal, Aberdeen Proving Ground, Maryland, under sponsorship of the U.S. Environmental Protection Agency. The report covers the period from March 1974 to September 1976.

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We wish to express our sincere gratitude to the Project Officers, R.P. McNamara, Chief, Toxicology Division, Edgewood Arsenal; and Charles Rogers, Solid and Hazardous Waste Research Division, EPA, for their advice and interest.

SECTION I

INTRODUCTION

Edgewood Arsenal has been engaged in the study of methods for the detoxification of toxic materials for many years. Continuing studies of this type are a fundamental part of Edgewood Arsenal's basic mission program. In 1974, based on this experience, Edgewood Arsenal and the Environmental Protection Agency entered into an interagency agreement to study methods for detoxification of a group of hazardous and toxic materials identified by EPA as Aldrin, Dieldrin, Endrin, DDD, DDE, DDT, Toxaphene and the PCB's and benzidine.

Studies of methods for deactivation of these compounds had involved only the aspects of chemical transformation, ignoring the question of toxicity of the resultant product. It was necessary, therefore, to review and evaluate existing chemical processes, characterize the resultant products, and assess their toxicity. These products will hopefully be nontoxic, and either have useful properties or be biodegradable.

For the second year effort the Toxicology Division was asked, in addition to the original group of hazardous materials, to assess techniques for detoxification and disposal of small amounts (5 gal, or 50 lb) of 35 common pesticides.

SECTION 7

CONCLUSIONS AND RECOMMENDATIONS

This report discusses some of the processes investigated as methods of eliminating hazardous and persistant materials from the environment. Two of the processes, sulfonation and incineration, show some promise and should be subjected to additional investigation. There are other processes, not yet studied, that may solve the problem. These should be studied.

Efforts should continue to seek new and more effective detoxification processes.

Assessments should be made for the toxicity of the products obtained and the safety of the processes used. Reaction products showing a low level of toxicity, water solubility or biodegradability should be studied further.

SECTION 3

LITERATURE SEARCH

A literature search was conducted in two phases. One, a machine search, made use of the computerized information retrieval facilities of MERC, Cincinnati. It emphasized the retrieval of existing information concerning the reaction of the subject compounds. The other phase was manual search of pertinent literature and included older literature published prior to that compiled in the computerized system. Also included was the chemistry of related compounds that might provide leads for new approaches to detoxification of the subject compounds. The search was completed in December 1974. A comprehensive report was prepared and submitted to EPA at that time.[4]

[4] A. Rednor and G. M. Steinberg, Assessment of Techniques for Detoxification of Selected Hazardous Materials, A literature search, Dec 1974.

SECTION 4

AMINE REACTIONS

CHEMISTRY

While searching the literature a patent was found for a process that seemed to offer promise.[4] The patent, held by the Millmaster-Onyx Corp., New York, N.Y., described reactions of PCB's with amines to give a variety of substituted amino-polychlorobiphenyls which were supposed to have some useful properties.

The Millmaster-Onyx Corporation was contacted to obtain information about the processes and samples for toxicological testing. The PCB reactions were no longer being considered, but two available samples were sent to us. They were analyzed for composition and purity then dissolved in suitable solvents so they could be used for animal toxicity studies. The glass-like solid AM 959 (from Aroclor 1260) was dissolved in water producing solution with pH of 6.4. The viscous AM 1078 (from Aroclor 1248) was dissolved in PEG-200 producing a solution with a pH of 8.5.

To determine whether the Millmaster-Onyx process reduced the toxicity of the PCB's, testing of the parent compounds was necessary. Aroclor is the trade name of PCB's manufactured in the U.S. solely by the Monsanto Chemical Company. An Aroclor is a complex mixture of isomers of PCB's. The various Aroclors are differentiated by a four-digit number, with the last two digits indicating the percentage of chlorine in the mixture. Aroclor 1242, for example, is a mixture containing 42% chlorine; Aroclor 1254 contains 54% chlorine. The Aroclors used in the Millmaster-Onyx reaction (1248 and 1260) are no longer being made. From the Monsanto Chemical Company samples of Aroclor 1242 and 1254 were obtained. These were used as comparative samples to judge the toxicity of the original Aroclors. It must be realized that the Aroclor 1242 and 1254 cannot be accepted as an absolute standard for comparison to Aroclor 1248 and 1260. It is hoped that they might provide a rough estimate for the toxicity of the originals.

[4] J.J. Merianos, E.G. Shay, B. Mead and A.N. Petrucci, U.S., 3,663, May 16, 1972. "N-(Halogenated Biphenyl)-Diethylenetriamines".

TOXICOLOGY

Comparative toxicity evaluations of Aroclor 1242, Aroclor 1254, diethylenetriamine and the two PCB reaction products AM 959 (from Aroclor 1260) and AM 1078 (from Aroclor 1268) were conducted. Intravenous LD50's were determined in mice, oral toxicity was estimated in rats, and skin and eye irritation evaluations following FDA procedures were performed in rabbits.[A] Results, as summarized in Table 1, show the PCB reaction products AM 959 and 1078 are 15 to 25 times more toxic to the mouse than the Aroclors to which they were compared. These reaction products are also more toxic (5 - 7 X) than diethylenetriamine in the mouse. Results in rats, though limited by small samples sized for testing, also show that the reaction products are more toxic than the Aroclors but about equally toxic to diethylenetriamine. Results of the irritation evaluations show the reaction products to be more irritating to the eye and equally irritating to rabbit skin as compared to the Aroclors. Diethylenetriamine was a severe irritant to both eyes and skin and by FDA standards would be classified as a primary irritant. These findings regarding irritation are not unexpected as it is known the amines, in general, are often culprits in this regard.

In addition to above mammalian toxicity evaluations, a 96-hr static bioassay in Bluegill Sunfish was performed using solutions of AM 959 and AM 1057. Concentration levels between 1.0 and 100 ppm of each product were used. All fish died within a few minutes at the 100 ppm level and at 4.8 ppm all fish died within 24 hours. The 96-hr TL50 for AM 1057 was found to be about 2 ppm and for AM 959 about 1.0 ppm. Results are shown in Table 2.

A See Appendix A

TABLE 1. TOXICITY STUDIES - MILLMASTER-ONYX PATENT

Compound	I.V. Mouse 24-hr LD50 mg/kg	Oral, Rat 1 gm/kg	Irritancy, Rabbit: Eye (0.1 ml/eye)	Skin
Aroclor 1242 (neat)	1342	1/10	0/6, Negative test non-irritant	P.I.I.=0.19*** non-irritant
Aroclor 1254 (neat)	975	0/10	1/6, Negative test non-irritant	P.I.I.=0.66 non-irritant
Diethylenetriamine (neat)	373	3/10, 3:1 min, over night, >24 <48 hr	6/6, Positive test irritant compound	P.I.I.=6.50 irritant compound
AR 959* (from Aroclor 1260)	48.7	4/10 >24 <48 hr(2) >48 <72 hr (2)	6/6, Positive test irritant compound	P.I.I.=8.79 (neat) P.I.I.=1.25
AR 1070** (from Aroclor 1348)	66.9	3/10 overnight (2) 48 hr (1)	6/6, Positive test irritant compound	P.I.I=2.73 (neat) P.I.I.=0.46 non-irritant

* Concentration = 45.8 mg/ml in water for I.V. mouse study
1.7/10 gal/ml in water for other studies

** concentration = 295.1 mg/ml in PEG 200 for I.V. mouse study
0.9995 gal/ml in PEG 200 for other studies

*** P.I.I = Primary Irritation Index, 25 = primary irritant

TABLE I. 96-hr TL_{50} STATIC BIOASSAY - BLUEGILL

Compound	Conc., ppm	Deaths in hours								
		1	2	5	7	8	24	32	48	96
AR - 959	0	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
	1.0	0/6	-	0/6	0/6	-	0/6	2/6	3/6	3/6
	2.4	0/6	-	2/6	4/6	-	6/6	-	-	6/6
	4.8	1/6	-	6/6	-	-	-	-	-	6/6
	7.5	6/6	-	-	-	-	-	-	-	6/6
	10.0	6/6	-	-	-	-	-	-	-	6/6
AR - 1857	0	0/6	0/6	-	-	-	-	-	-	0/6
	1.0	0/6	-	-	-	-	-	-	-	0/6
	2.4	0/6	-	-	-	-	1/6	3/6	5/6	5/6
	4.8	0/6	-	3/6	4/6	-	6/6	-	-	6/6
	7.5	4/6	6/6	-	-	-	-	-	-	6/6
	10.0	6/6	-	-	-	-	-	-	-	6/6

SECTION 5

CATALYTIC PROCESSING

CHEMISTRY

As part of the interagency agreement between EPA and Edgewood Arsenal (EA), compounds from other sources were to be submitted to EA for toxicological characterization. Dr. Wilmer Kranich of Worcester Polytechnic Institute, studying catalytic decomposition of chlorinated hydrocarbons, was a principal source of compounds for evaluation.

TOXICOLOGY

Some difficulty was encountered in evaluating these compounds primarily because the samples were too small and/or insoluble for toxicity testing. In spite of these difficulities attempted were made to evaluate their toxicity. To date, a total of 24 compounds submitted by Dr. Kranich have been evaluated in the mouse. Five are products from Aroclor, eleven are products from DDT, two are products from Toxaphene, and six are products from Dieldrin. Results of these tests are given in Table 3.

These results are very difficult to interpret as solutions received were not characterized by composition or concentration. As a consequence, results could only be expressed in volume of material injected per kilogram of body weight. Other materials were received as solids and could therefore be weighed and the weight of material per kilogram of body weight is used. There is no way to make a direct comparison between the two expressions until something is known about the solutions tested (specific gravity, concentration) that would allow translation of volume units to weight units.

For the Aroclor compounds, except for compound WPI-2, it would appear that the products from Dr. Kranich's catalytic processing are about as toxic as the reference Aroclor tested. One, WPI-1, is almost half as toxic (0.65 ml/kg) and one, WPI-13, twice as toxic (0.22 ml/kg).

TABLE 1. TOXICOLOGICAL STUDIES OF COMPOUNDS PRODUCED BY WORCESTER POLYTECHNIC INSTITUTE

Sample Number	Starting Material	14-day [illegible]
Reference	Aroclor	0.37 (0.30-0.45) ml/kg
WPI - 1	"	0.65(0.63-0.68) ml/kg
WPI - 3	"	0.48(0.40-0.59) ml/kg
WPI - 2	"	114.9 (104.7-126.2) ml/kg
WPI - 12*	"	0.37(0.34-0.42) ml/kg
WPI - 13	"	0.22(0.20-0.25) ml/kg
Reference	DDT	76.8(68.1-86.7) mg/kg
WPI - 4	"	not tested-bottle broken
WPI - 5	"	196.4(155.5-247.8) mg/kg
WPI - 6	"	1.0(0.95-1.05) ml/kg
WPI - 7	"	1.42(1.29-1.54) ml/kg
WPI - 8	"	0.071(0.064-0.079) ml/kg
WPI - 9	"	198. (164-242) mg/kg
WPI - 10	"	162. (144-182) mg/kg
WPI - 11	"	351. (320-384) mg/kg
WPI - 22	"	0.40 (0.37-0.43) ml/kg
WPI - 23	"	0.79(0.73-0.86) ml/kg
WPI - 24	"	0.56(0.56-0.56) mg/kg
Reference	Toxaphene	20.5(19.6-21.4) mg/kg
WPI - 14	"	0.39(0.33-0.45) ml/kg
WPI - 15	"	0.14(0.12-0.16) ml/kg
Reference	Dieldrin	10.5(9.3-11.8) mg/kg
WPI - 16	"	0.29(0.26-11.8) ml/kg
WPI - 17	"	0.35(0.31-0.40) ml/kg
WPI - 18	"	0.11(0.10-0.14) ml/kg
WPI - 19	"	0.40(0.37-0.43) ml/kg
WPI - 20	"	0.30(0.27-0.33) ml/kg
WPI - 21	"	136. (117-160) mg/kg

* Supernatant fluid

For DDT residues (expressed in mg/kg) compared with the reference compound, it appears the residues WPI-5, 9, 10 and 11 are approximately 2 to 4.5 times less toxic than reference DDT. No other comparisons are possible at this time. No comparisons are possible for the fusaphene compounds.

For the Dieldrin compounds the reference compound can only be compared with WPI-21. In this case WPI-21 is about 11 time less toxic than Dieldrin. A comparison with Dieldrin cannot be made for the other products, WPI-16 variation being a factor of 4, etc.

SECTION 6

SULFONATION

CHEMISTRY

Background

Since compounds were not being received from outside sources, in June 1974 EPA suggested that Edgewood Arsenal might try chemical processes for detoxification of the chlorinated hydrocarbons. Edgewood Arsenal personnel have, since September 1974, been working on this problem.

The first attempts were to convert DDT to water soluble compounds. The hope was that this would prevent accumulation in the food chain.

DBA has been found to be a metabolite of DDT in several animal species and is excreted in the urine various species including man. It is, therefore, not expected to accumulate in the food chain.

DBA is obtained by the basic hydrolysis of either DDT or its elimination product 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) via alcoholic potassium hydroxide or in ethylene glycol containing barium hydroxide. The metabolism and fate of DBA is unknown to us.

DDT is notable for its lipophilicity and reluctance to enter the aqueous phase. In order to cause reaction between DDT and nucleophilic reagents, a suitable reaction medium is necessary. To accomplish this non-aqueous solvents, bi-phasic aqueous-organic solvent mixtures and aqueous solutions containing detergents or phase-transfer catalysts were considered.

As a class, nucleophilic reactions are base catalyzed. In the presence of even mild base, DDT eliminates HCl to produce DDE. For this reason, DDE is usually a product of the nucleophilic reaction of DDT and is resistant to further nucleophilic attack.

The conversion of DDT to DBA by phase-transfer catalysis was attempted in a mixture of DDT, toluene, sodium hydroxide, water and tetrabutylammonium iodide by heating to 88°C for 6 hours. The DDT was completely converted to this dehydrochlorination product DDE. No DBA was detected.

DDA was successfully prepared from DDT by the action of potassium hydroxide in diethylene glycol at 130°C for 6 hours. The yield, however, was not quantitative and the product was fairly toxic (84 mg/kg, iv mouse).

The recently reported electrochemical oxidation of DDE to DBA is of considerable interest. A postulated mechanism is outlined in Figure 1.

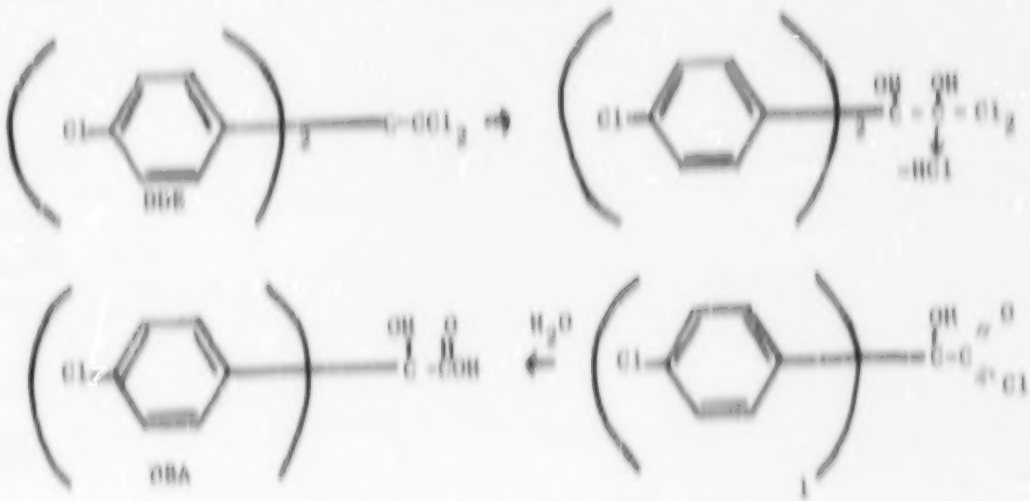


Figure 1. Postulated mechanism for electrochemical oxidation of DDE to DBA.

Several attempted conversions of DDT to DBA using basic potassium permanganate with phase-transfer conditions according to the following scheme results in the conversion of DDT to DDE with little or no oxidation taking place.

The attempted reaction of DDT with sodium thiosulfate by phase-transfer catalysis using benzene as the organic solvent and tetrabutylammonium iodide as the catalyst yielded the quantitative return of DDT.

The fusion of molten sodium hydroxide and DDT yielded a product that was not water soluble (probable DDE). The fusion of molten sodium thiosulfate with DDT produced essentially the same results except two phases were visible in the melt. The product was apparently unreacted DDT.

The attempted reaction of DDT with sodium thiosulfate in an aqueous-detergent mixture also yielded only unreacted DDT. DDT was heated in an aqueous solution of sodium thosulfate to which 25% (V:V) of Tween 60 (a cationic detergent) had been added. Although the DDT appeared to dissolve

at 60° to 70°C; no reaction occured and DDT was recovered quantitatively.

In attempts to prepare sulfate derivatives of DDT and DDE, several experiments were performed. DDT was added to an aqueous solution of sulfuric acid and cetyltrimethylammonium bromide. Heating to the boiling point did not cause the DDT to dissolve. In other experiments, DDE was heated with concentrated sulfuric acid at 80°C, at 150°C and in a refluxing solution of sulfuric acid in DMF. In none of these instances did reaction occur.

These reactions demonstrate properties of DDT that govern its behavior in the environment. Although these attempts were intended to solubilize and degrade DDT, they clearly demonstrated, instead, that this insecticide can exist under certain reaction conditions even more severe than those encountered in the environment. Although DDT is reactive as an electrophile, its solubility is such that it can only rarely contact nucleophilic reagents. When this does occur, elimination of HCl readily yields DDE which is more resistant to further attack.

Sulfonation Reaction

DDT, DDE, DDD

A report of a water soluble sulfonation product of undetermined structure was found in the patent literature. Investigations have been conducted for sulfonation of DDT, DDE and DDD under a variety of conditions. Results are summarized in Table 4.

TABLE 4. SULFONATION OF DDT, DDE AND DDD

Substrate	Purity	ISO_3	Temperature[A]	Product[AA]
DDT	99%	10	S.B.	U
DDT	99%	30	150°	U
DDT	99%	20	S.B.	S
DDT	99%	20	R.T.	S
DDT	Tech[AAA]	20	S.B.	S&UK
DDE	99%	10	S.B.	S
DDE	99%	20	S.B.	S
DDE	99%	20	S.B.	S&U
DDD	Tech[AAAA]	30	150°	UK
DDD	Tech[AAAA]	20	R.T.	S&UK
DDD	Tech[AAAA]	30	S.B.	U&UK

[A] S.B., Steam Bath; R.T., Room Temperature
[AA] U, Unsymmetrical; S, Symmetrical; UK, Unknown
[AAA] Contains 20% o,p' DDT [AAAA] Contains 25% o,p' DDD

When DDT is treated with fuming sulfuric acid containing 20 percent free SO_3, overnight at room temperature or for 5 hours on a steam bath, 2,2-bis(3-sulfo-4-chlorophenyl)-1,1-trichloroethane (I) is formed. (Fig. 2) Upon treatment with base during workup, hydrogen chloride is eliminated as with DDT to form 2,2-bis(3-sulfo-4-chlorophenyl)-1,1-dichloroethylene disodium salt (II). When DDT is treated with fuming sulfuric acid containing 30 percent free SO_3 on the steam bath or at 150°, product isolated is 2-(3,5-disulfo-4-chlorophenyl)-2-(4-chlorophenyl)-1,1-dichloroethylene disodium salt (III).

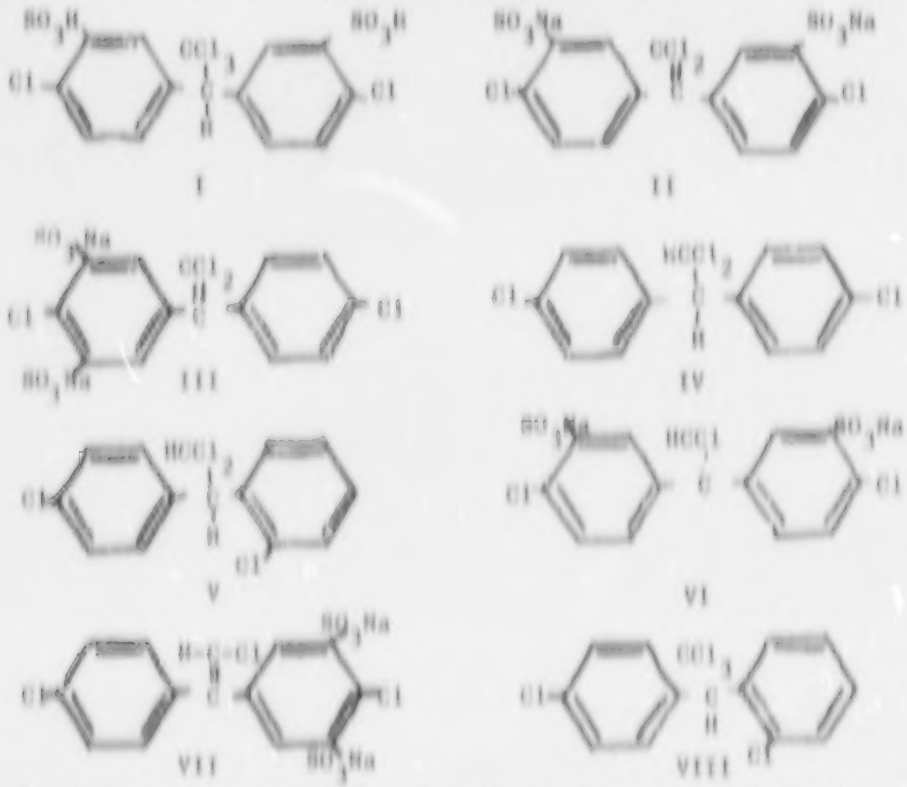


Figure 2. Some parent compounds and sulfonated products of DDT and DDD

In our DDD sulfonation experiments, technical grade DDD was used. It is a mixture of approximately 75 percent p,p' DDD (IV) and 25 percent o,p' DDD (V), and yields a mixture of products upon sulfonation. The p,p' DDD appears to be sulfonated similarly to DDT in that treatment at room temperature with 20 percent SO_3 oleum[A] leads to the symmetrically substituted product (VI) and sulfonation with 30 percent SO_3 oleum leads to the unsymmetrically substituted product (VII). In both cases, the o,p' isomer (V) gave rise to a product or products whose structure(s) could not be determined by Nuclear-Magnetic resonance (NMR) while in mixture VI or VII. Sulfonation with 30 percent SO_3 oleum at 130° gave a more complex mixture. Its NMR spectrum was too complex to interpret.

[A] A heavy oily strongly corrosive solution of sulfur trioxide in anhydrous sulfuric acid.

When technical DDT containing 20 percent of o,p' isomer(VIII)was sulfonated with 20 percent SO_3 oleum on a steam bath, a mixture of the symmetrically substituted product,(III) and other unknown product or products were found.

The sulfonation of pure, DDE, both with 20 and 30 percent fuming sulfuric acid, on a steam bath, led to the symmetrical product. However, a recent run has produced a mixture of 76 percent of the symmetrical isomer and 24 percent of the unsymmetrical isomer. This result is not yet understood.

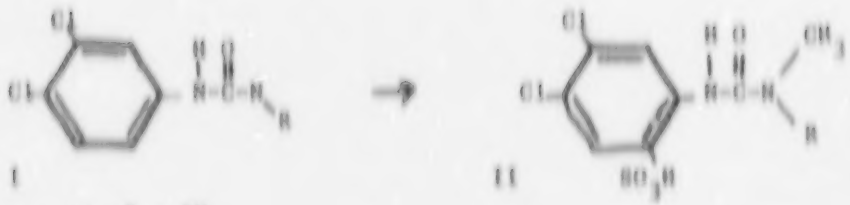
Polychlorinated Biphenyls (PCB's)

The polychlorinated biphenyls(PCB's) are mixtures of biphenyls which have been chlorinated to varying degrees. Theorethically 210 different chlorination products can exist. In the U. S., the Monsanto Co. produces these mixtures under the trade name Aroclor. We have undertaken the sulfonation of Aroclor 1242 and 1254 (products containing 42% and 54% chlorine by weight, respectively). Both Aroclor 1242 and Aroclor 1254 when heated at 95° with fuming sulfuric acid for 5 hours yield highly water soluble ammonium sulfonates along with sulfur containing a material which is insoluble in acid, base, water, and a wide variety of organic solvents. It is probably a sulfonation product of highly chlorinated PCB's. Because of the higher toxicity of the water soluble products (417 mg/kg and 247 mg/kg, i.v. mouse, for PCB 1242 and 1254 sulfonates respectively) further investigations of these products has not been continued.

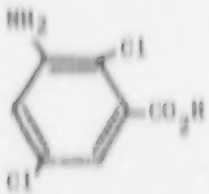
Other Chlorinated Hydrocarbon Insecticides (See Figure 3 below)

Diuron (Ia) and Linuron (Ib) are readily converted to 3-(4,5-dichloro-2-hydroxysulfonylphenyl)-1,1-dimethylurea (IIa) and 3-(4,5-dichloro-2-hydroxysulfonylphenyl)-1-methoxyl-1-methylurea (IIb), respectively. Diuron sulfonate was isolated as the free acid and Linuron sulfonate as the ammonium salt.

Chloraben (3-amino-2,5-dichlorobenzoic acid) III, was treated for 5 hours at 95° with 30 percent oleum. The reaction was worked up to yield the water soluble ammonium salt of the sulfonation product. The NMR spectrum shows a singlet in the aromatic region but the site of sulfonation could not be determined by this technique.



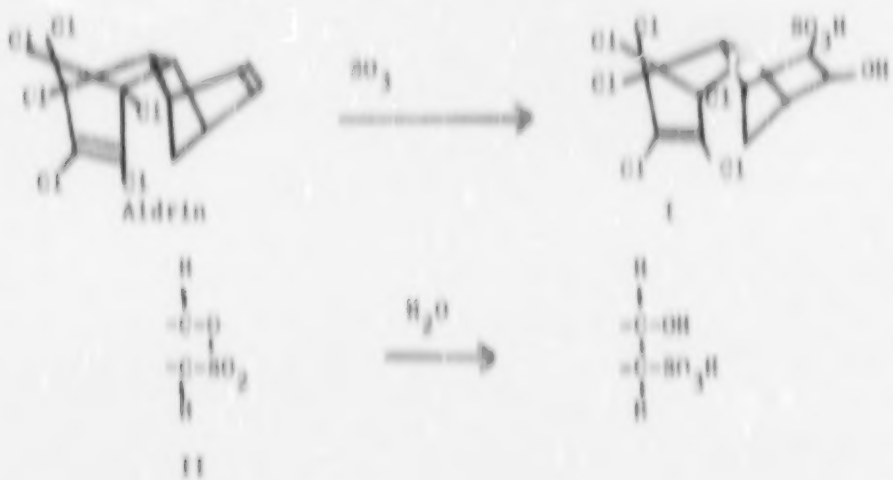
a: R = CH_3
b: R = OCH



III

Figure 3. Sulfonation of three chlorinated hydrocarbon insecticides.

Aldrin, when heated at 100° with 30 percent fuming sulfuric acid, yields what NMR and mass spectrometry suggest to be the hydroxy sulfonate I. The primary product of the sulfonated reaction is probably the sultone, II, which yields the hydroxy sulfonate upon hydrolysis. (See Figure 4 below)



Aldrin

I

II

Figure 4. Sulfonation of Aldrin

When Endrin was treated with fuming sulfuric, an exothermic reaction ensued. The product from this reaction is sec.- endrin, a metabolite of Endrin known to form under acidic conditions. (See Figure 5 below)

Endrin

Figure 5. Attempted sulfonation of Endrin

Chlordane was recovered almost quantitatively after treatment with 30 percent oleum on a steam bath for 5 hours. Treatment with alcoholic potassium hydroxide led to the formation of the diene.(See Figure 6 below)

Chlordane Diene

Figure 6. Dehydrohalogenation of Chlordane

Attempted sulfonation of the diene resulted in the formation of a material of unknown composition which contained no sulfur.

Workup of the reaction mixtures of heptachlor and 30 percent fuming sulfuric acid with ammonium hydroxide led to the recovery of ammonium chloride but no sulfonation product.

Toxaphene on treatment with 30 percent oleum did not yield a sulfonated product.

2,4-Dichlorophenoxyacetic acid (2,4-D) reacted exothermally with charring when treated with 30 percent fuming sulfuric acid and no clean product was isolated.

An estimate of the approximate per pound cost for detoxification of small and large amounts of pesticide using the sulfonation process is enclosed as appendix B.

TOXICOLOGY

Acute

Acute 96-hr toxicity screening tests were conducted using DDE and DDE disulfate sodium salts against bluegills. Six concentrations were used for each compound: 100, 10, 1, 0.1 and 0.001 mg/l for DDE. For the DDE disulfate the lowest concentration was changed to 0.005 mg/l. The DDE was dissolved in acetone. Control tanks were used with acetone equal to the in the DDE solution. Four fish were exposed at each concentration. Table 5 gives the results of this study. The sulfonate produced no mortalities while the unconverted DDE was toxic to fish at concentration for 1 mg/l and up. Table 6 shows the I.V. mouse LD50's for reference samples and some of the sulfonated compounds

TABLE 5. 96 Hr TOXICITY SCREENING SULFONATE

Compound	Species	Toxicity
DDE disulfate sodium salt	Bluegills	No mortality at concentration from 0.005 to 100 mg/l
DDE (in acetone)	Bluegills	[A] 50% mortality at concentrations of 10 and 100 mg/l 100% mortality at concentration of 1 mg/l
Acetone Control	Bluegills	No mortality at concentrations equal to those used with the DDE

[A] Lower mortality at higher concentrations possible caused by DDE coming out of solution in water.

TABLE 5. I.V. MOUSE LD50 (mg/kg) SULFONATES

Compound	LD50 24 hr	LD50 14 day
Aldrin (Ref. Sample)	15.65	14.31
Aldrin(Sulfonate)	147	same
Aldrin (Sulfate Na Salt)	800.8	702.1
Aroclor 1242 (Ref. Sample)	925	813
Aroclor 1242 (Ammonium Sulfonate)	417	same
Aroclor 1254 (Ref. Sample)	1214	608
Aroclor 1254 (Ammonium Sulfonate)	288.8	246.6
Chlordane (Ref. Sample)	159.6	127.5
Chlordane (from Base)	171.5	167.6
DDT (Ref. Sample)	80.7	76.8
DDE (Disulfonate Sodium Salt)	648.2	same
DDD (Disulfate Sodium Salt)	1118.5	same

Eggshell Study

The effect of decreasing eggshell thickness and weight due to DDT or DDE ingestion has been shown to occur both naturally and in controlled laboratory experiments. Morphologic alterations in the eggshell gland were seen in mallard ducks (Anas platyrhynchos) fed 75 ppm DDT for 6 weeks prior to egg production in experiments conducted in this laboratory. DDE has been shown to cause inhibition of Ca ATPase in in-vitro experiments. Ca ATPase is an enzyme responsible for transport of eggshell calcium. A study was undertaken to determine the effects of DDT, DDE and their sulfonated derivatives on eggshell production. To be examined were (1) eggshell measurements including weight, length, width, thickness, and the ratio of weight/length x width; (2) light and electron microscopy of eggshell gland, liver and kidney and (3) Ca ATPase activity.

Young adult mallard ducks obtained from a local supplier were randomly assigned to cages, 3 females and 1 male per cage, and maintained on commercial poultry laying mash. After a 2 month acclimatization period egg production was induced by regulating the photoperiod. When peak egg production was reached feeding continued with either 10 or 50 ppm of DDT, DDE, DDT-SO$_3$ or DDE-SO$_3$ added to the same poultry laying mash. Four groups were fed the normal diet. Eggs were collected daily from each group for 30 days. The contents were removed from the eggs and the hollow shells were

air dried and stored until measurements were made. A total of 634 eggs were measured for (1) weight (grams), (2) overall length and width (cm), and (3) shell thickness (mm). Four measurements were made around the girth of each egg.

One hen from each group was sacrificed after 1 week of feeding the compounds. The remaining hens were sacrificed after 30 days. Sections of eggshells gland, liver and kedney were fixed in 4% formaldehyde, 1% glutaraldehyde and 200 millimole PO_4 buffer for evaluation by light and electron microscopy. Additional sections of eggshell gland were collected and processed to obtain a microsomal fraction for Ca ATPase determination. Sections of fat, brain and eggshell gland were taken from each group to be analyzed for pesticides by gas chromatography. Statistical analysis of all data was done by Least-Squares and Maximum Likelihood General Purpose Program.[A]

In all statistical analyses performed, no differences were seen between dose levels of the compound fed and the 10 and 50 ppm groups are considered together. Figure 1 shows the results of eggshell thickness measurements over the 30 day experimental period. There were no statistical differences in mean thickness of control eggs between the days measured; the mean value was .401 mm. As expected, the thickness of the eggs from ducks fed DDE was significantly reduced ($p<.01$) at day 1 and remained thinner for the entire 30 day period. The eggs from the ducks fed DDT remained as thick as control eggs until day 14 but were significantly thinner ($p<.01$) for the remainder of the experimental period. The DDT-SO_4 and DDE-SO_4 ducks produced eggs which were identical in thickness measurements and were grouped together. The sulfonated groups laid eggs which were the same thickness as controls except on day 18. Where there was significant difference ($p<.01$). The thickness returned to control levels at day 27 and remainder of the experiment.

Figure 2 shows the R-values[AA] obtained for eggshells from ducks fed DDT, DDE, sulfonated derivatives and control diets. The purpose of the R-values is to correct for eggshell weight in relation to size. A significant reduction is shown in the R-values for eggshells from DDT and DDE fed ducks when compared to controls. There was no significant difference between the R-values obtained from the sulfonated and control eggshells. The DDT-SO_4 and DDE-SO_4 values did not vary significantly and were considered as one group. The graph in figure 2 shows a sharp decline at day 18 because heat drying was used instead of air drying from this day until the end of the experiment. Since all groups were treated in the same manner, the relationship between the groups remained constant. The weights of the eggshells followed the same pattern as thickness measurements. Significant differences were seen in length and width measurements of eggshells from the DDE-SO_4 and DDT fed ducks.

[A] I wish to acknowledge the assistance in computer programming and statistical analysis of the data by Mr. Elden Leighton and Dr. Jerry F. Hardisty.

[AA] $R = \frac{\text{Weight}}{\text{Length} \times \text{Width}}$

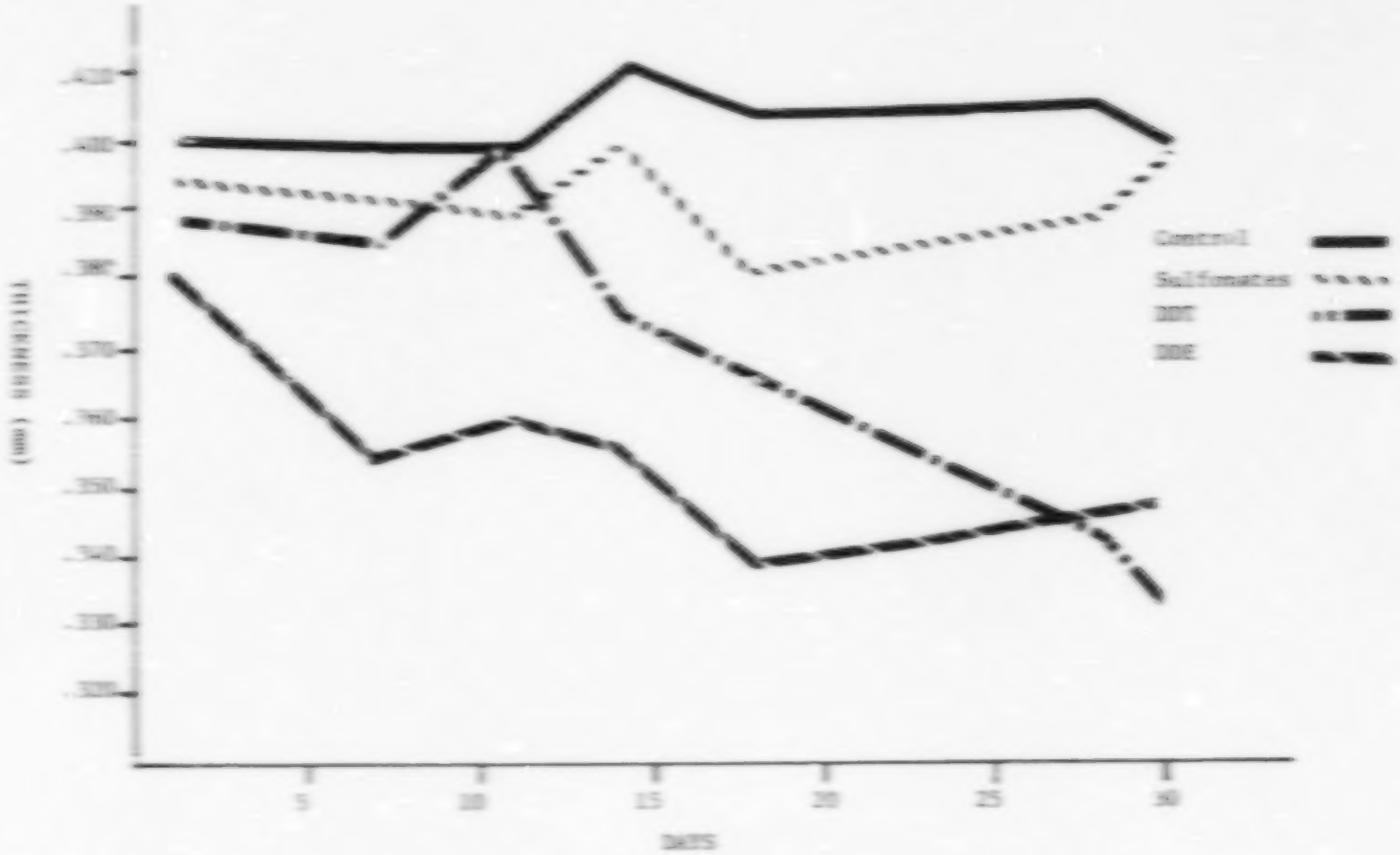


Figure 1. Mean Eggshell Thickness From Ducks Fed DDT, DDE, Sulfonates and Control Diets

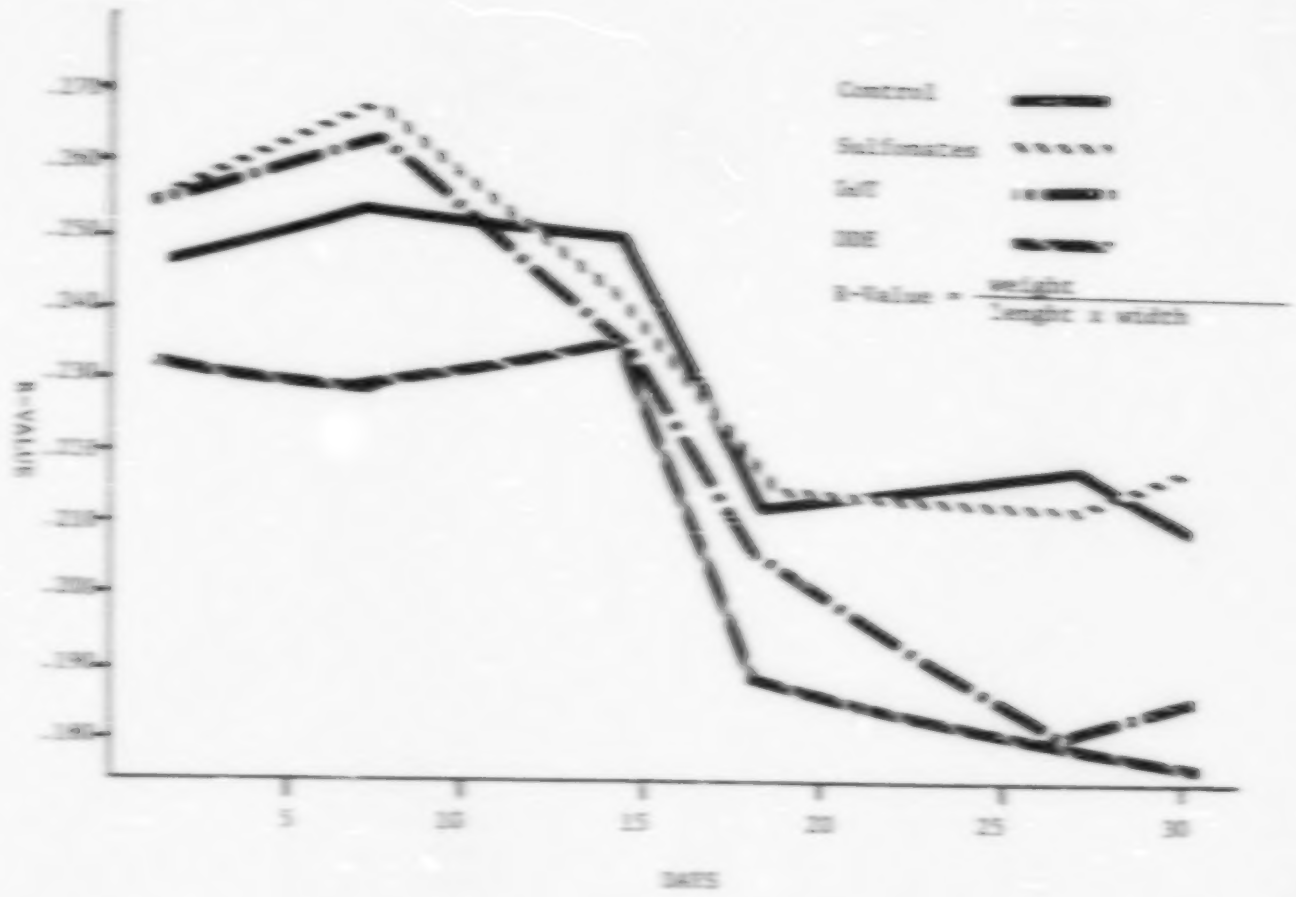


Figure 1. Mean B-Values for Eggshells From Ducks Fed DDT, DDE, Sulfonates and Control Diets

These differences were corrected for by using R-values.

The tissues collected for electron microscopy are being processed and results will be available soon. All samples collected for Ca ATPase analysis are being stored at -60°C. In vitro analysis of DDT, DDE and sulfonated compounds will be performed.

The results of this experiment show to that while DDT and DDE fed at levels of 10 and 50 ppm cause significant alterations in eggshell measurements such as thickness, weight, and R-values, their sulfonated derivatives do not share these characteristics. Thickness measurements show that while the sulfonated compounds cause a reduction of thickness at day 18 this decreased thickness is not as severe as that caused by DDT or DDE and is transitory effect.

The R-values show that DDT and DDE are potent agents in causing a reduction of the weight to size of eggshells but the sulfonated compounds cause no change. The final portion of this study, the electron microscopic study of eggshell gland, liver and kidney and the Ca++ ATPase measurements should be of additional value in determining whether these sulfonated derivatives share some of the toxic properties of the parent compounds.

BIODEGRADATION STUDIES

One of the hoped for goals of any pesticide conversion product is that it be biodegradable.

Several experiments have been carried out to determine if some of the water soluble sulfonates described above are biodegradable. In our first experiment, sodium salts of unsymmetrical disulfonic acid, (derived from DDT), symmetrical disulfonic acid (derived from DDE), and an undefined mixture of sulfonation products derived from DDD, all at a concentration of 98 mg/l, were incubated at room temperature in a basal mineral medium which was inoculated with 5 volume percent of raw sewage from the Bod Run Sewage Treatment Plant of Harford County, Md. Substarate concentrations were determined spectrophotometrically on a periodic basis. After seven weeks no change was observed.

Since soil contaiminated with DDT might contain microorganisms capable of utilizing sulfonated DDT derivatives as a source of carbon, additional experiments were performed. Such a soil was obtained from the manufacturing area of Pine Bluff Arsenal throught the courtesy of Robert Donald, Ecological Research Office, Biomedical Laboratory. The sulfonates derived from DDT and DDE (150 mg/l) were incubated in the medium of which two weight percent of

soil was added. The samples were shaken continuously on a rotary shaker. Aliquots were periodically taken, centrifuged, and the level of the pesticide remaining in the supernatent was determined. After eight weeks, no change was observed.

Recent investigations have indicated, however, that mixed bacterial cultures can mineralize some chlorinated aromatic compound which do not sustain microbial growth, if they are supplied with another source of carbon and energy, the so called "co-substrate enrichment technique." In our experiments using this method, we chose as substrates, the symmetrical and unsymmetrical sulfonates of DDT, the sulfonates of diuron, linuron, and as positive controls, 2,4-D and p-chlorobenzoic acid, both of which are known to undergo microbial degradation. The chlorinated substrates were added to the medium at a concentration of 25 mg/l. Each compound under study was incubated both with glucose as a co-substrate (500 mg/l) and without glucose. The cultures were inoculated with 2.7 volume percent of sewage from the Sod Run Plant and incubated at ambient temperature on a rotary shaker. Levels of substrates were determined as above. After 24 hours, all systems to which co-substrate had been added had become quite turbid indicating bacterial growth. After 20 days incubation, 47 percent of the original 2,4-D absorbance at 283 mm remained in the culture; no co-substrate, and no absorbance was observed in the culture with co-substrate.

Both p-chlorobenzoic acid cultures has about 65 percent of their original absorbance at 267 mm. No change was observed in the other systems.

SECTION 7

INCINERATOR RESIDUES

Twenty-four incinerator residue samples, produced by Midwest Research Institute (Table 7), were submitted by EPA for toxicological testing. These samples were insoluble in all commonly used solvents so I.v. mammalian evaluations could not be run. Since standard methods could not be used, 11 of the residues were pulverized, placed in fish tanks and screened for 96-hr toxicity to bluegills. Most of the material settled out after 24-48 hours with the highest concentration (1000 mg/l) remaining quite turbid after 96 hours. Of all samples only Captan run #148 horizontal stack residue produced mortality below 1000 mg/l. The 96 hr TL50 for this sample was 320 mg/l. All other samples appeared to be non-toxic at concentration up to 1000 mg/l.

TABLE 7. MIDWEST RESEARCH INSTITUTE SAMPLES

Test No.	Pesticide	Residue Sample(s)
44[a]	DDT Dust	Primary and Secondary Chambers
45	DDT Dust	Primary and Secondary Chambers
61[a]	Aldrin Granules	Primary and Secondary Chambers
64	Aldrin Granules	Primary and Secondary Chambers
86	Picloram Pellets	Primary Chamber (Ground)
87	Picloram Pellets	Primary Chamber
104[a]	Malathion Dust	Primary and Secondary Chambers
105	Malathion Dust	Primary and Secondary Chambers
122	Toxaphene Dust	Primary and Secondary Chambers
124[a]	Toxaphene Dust	Primary and Secondary Chambers
146	Captan Dust	Primary and Secondary Chambers
148[aa]	Captan Dust	Primary and Secondary Chambers
149	Zineb Dust	Primary and Secondary Chambers
150	Zineb Dust	Primary and Secondary Chambers
170	Mirex Bait	Primary Chamber
172	Mirex Bait	Primary Chamber
After 148[aa]	Captan Dusts Tests	Horizontal Stack Residue
After 150	Zineb Dust Tests	Scrubber 1 Residue
After 150	Zineb Dust Tests	Vertical Stack Residue
After 126	Toxaphene Dust Tests	Scrubber 1 Residue
After 50	DDT Dust Tests	Scrubber 1 Residue
After 126	Toxaphene Dust Tests	Horizontal Stack Residue

Table 7. continued

Test No.	Pesticide	Residue Sample(s)
After 106	Malathion Dust Tests	Horizontal Stack Residue
After 50^	DDT Dust Tests	Horizontal Stack Residue

^Single sample tested - these samples were tested for toxicity in fish.
^^2 samples tested - these samples were tested for toxicity in fish.

APPENDIX A

DESCRIPTION OF ACUTE TOXICITY STUDIES

ACUTE LD50 DETERMINATION IN THE MOUSE

Male mice from the Edgewood Arsenal colony are used in these tests. All animals are acclimated to the test environment at least 24 hours before test. Ten animals are tested per dose level and a minimum of 4 logrithmically spaced dose levels are used in an effort to produce 0% mortality, 100% mortality and two partial mortality fractions. All injections are made into the lateral tail vein. LD50's with 95% confidence limits are calculated for 24 hours results as well as for 14 day observation results. Where possible doses are expressed in mg/kg values. When solutions of unknown characterization is tested doses are expressed in ml/kg.

ORAL TOXICITY TESTS

Rats from the Edgewood Arsenal colony are used in these tests. Except for the species and route of administration (stomach tube) tests are conducted in a manner similar to that described for mice. Where insufficient sample amounts are available to perform a complete test a selected dose or two of material are given to the animals and the result out toxicity response compared.

SKIN IRRITATION (CFR, TITLE 21, PARA 191.11)

Primary irritation to the skin is measured by a patch-test technique on the abraded and intact skin of albino rabbits whose backs were clipped free of hair 24 hours prior to compound application. Six animals are used for each material tested. The test compound, as a liquid (0.5 ml) or a solide (0.5 gm) is applied under a gauze patch to the abraded and intact skin of each animal. Immediately after compound application the animals trunk is wrapped withimpermeable plastic to retard evaporation. After 24 hours of exposure the plastic and gauze are removed from each animal and the resulting reactions to the skin are evaluated and scored according to the FDA

procedure. See Table A-1. Readings are again made 72 hours after initiation of exposure.

TABLE A-1. FDA SCORING TABLE FOR SKIN IRRITATION

Skin Reaction	Value
Erythema and Eschar Formation	
No erythema	0
Very slight erythema	1
Well defined erythema	2
Mod to severe erythema	3
Severe erythema/eschar	4
Edema Formation	
No edema	0
Very slight edema	1
Slight edema	2
Moderate edema	3
Severe edema	4

To establish the primary irritation index score (PII), readings obtained for skin reactions against abraded and intact skin are added together for both the 24-hour readings for both erythema/eschar formation and for edema formation. Thus, a total of four values for erythema/eschar and four values for edema are obtained. The total of the eight values are added together and divided by four to give the primary irritation index score for the compound. When the PII score equals or exceeds a value of 5, the compound is rated as a primary skin irritant. No ratings are provided for this test by the FDA for values to less than 5 though it is clear that compounds having a PII score between 3 and 4.9 are significant skin irritants but would not, by definition, be classified as a primary skin irritant.

EYE IRRITATION TEST (CFR, TITLE 21, PARA, 191.12)

Six albino rabbits are used to evaluate eye irritancy potential of each test material. Both eyes of test animals are examined prior to testing using fluorescein and UV light to assure that only animals without eye damage or irritation are used for testing. All animals are maintained in facilities free of sawdust, wood chips, or other type bedding material that may produce eye irritation.

Each test animal is given 0.1 ml of liquid compound or 100 mg of solid compound into one eye. The other eye serves as a comparative control. The test substance is placed into a cup formed by gently pulling the lower lid out. After instillation of the test compound the eye lids are held together for approximately five seconds to insure thorough distribution. Test compounds are not washed out of the eye until 24-hours after instillation at which time they are flushed thoroughly with copious quantities of distilled water.

Eyes are examined, graded, and recorded for ocular reaction at 24, 48 and 72 hours after compound instillation. After recording the observations at 24 hours, all eyes are further examined after instillation of fluorescein dye using ultraviolet light.

The test is considered positive if four or more animals in the test group exhibited a positive reaction. If only one animal exhibits a reaction the test is regarded as negative. If two or three animals exhibit a positive reaction the test is repeated using another group of six animals. In the second test if three or more animals exhibit a positive reaction the test is positive. If one or two animals exhibit positive reaction the test is repeated again. If none of the animals exhibit positive irritation the test is negative. In the third test if one or more animals exhibit a positive response the test is positive and if no positive responses are seen the test is negative.

Grades for ocular lesions as defined by the FDA in CFR, Title 21, para. 191.12 are shown in Table A-2.

TABLE A-2. GRADATION OF EYE EFFECTS

	Grade
Cornea (C)	
No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible	1[A]
Easily discernible translucent areas, details of iris slightly obscured	2
Nacreous areas, no details of iris visible, size of pupil barely discernible	3
Iris (I)	
Normal	0
Markedly deepend folds, congestion, swelling moderate circumcorneal injection (any of these or combination of any thereof), iris still reacting to light (sluggish reaction is positvie)	1[A]
No reaction to light, hemorrhage, gross destruction (any or all of these)	2
Conjunctival Redness (R) (refers to palpebral and bulbar conjunctivae excluding cornea and iris)	
Vessels normal	0
Some vessels definitely injected	1
Diffuse, crimson red, individual vessels not easily discernible	2[A]
Diffuse beefy red	3
Chemosis (CH)	
No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2[A]
Swelling with lids about half closed	3
Swelling with lids more than half closed	4

[A] Indicates lowest grades considered positive under Section 191.12 of the Federal Hazardous Substances Labeling Act Regulations.

APPENDIX B

APPROXIMATE OPERATING COSTS OF PROPOSED PROCESS

We have applied commodity costs taken from current Chem. Marketing Reporter and current labor costs in Chemical Process Technology Branch to the process information you gave us on 25 Feb. From this, we arrived at estimates for the process of chemical destruction of DDT (representative of chlorinated aromatic hydrocarbons) in batch quantities of 50 and 2000 lbs per day.

The estimated costs are for rough labor and material only. Not included are facility acquisition costs or the costs of any further disposition of the reaction products.

PROCESS DATA USED

1. 30% fuming sulfuric per kilo DDT = 1.5 liter
2. NaOH equivalent to all of acid used.
3. Digestion of DDT in acid for 5 hrs at 95°C.
4. Neutralization of reaction mass with aqueous NaOH.

COMMODITY DATA

1. Acid = $53.25/ton (less than carload lots) for 100% acid X 1.065 for 20% fuming (30% not listed).
2. NaOH = 76% beads $15.00/100 lbs.

MATERIAL REQUIREMENTS AND COST PER POUND OF DDT

1. 2000 lbs DDT equivalent to 910 Kg, equivalent to 1355 l. acid
   1355 l. acid equivalent to 375 gals
   375 gals :: 1.9 sp. g. (approx) = 5940 say 6000 lbs
   6000 lbs :: 1 at $53.25 X 1.05 $/ton = $167 = $0.084/lbs DDT

2. 30% fuming H_2SO_4 ≈106.7% total H_2SO_4

1.067 X 6000 X 80/98 ≈ 5226 lbs NaOH (100%)
5226/0.76 = 6876 say 6900 lbs 76% NaOH
6900 lbs at $15.00/100 lbs = $1035
$1035/2000 lbs DDT = $0.52/lbs DDT

3. Total material requirements and costs:

Fuming sulfuric acid	= 1 lbs/lb DDT	= $0.083/lb DDT
76% NaOH	= 1.63 lb/lb DDT	= 0.52/lb DDT
		$0.63/lb DDT

Round off to $0.75/lb DDT to account for steam, water and power.

ESTIMATED LABOR COSTS

50 lbs DDT/day basis - 8 man hours

2000 lbs DDT/day basis - 16 man hours

At prevailing Cml Proc Tech stabilized labor rate of $30.38/hr, labor costs are:

50 lbs/day rate = 2.3 say $2.50 = $5.00/lb DDT

2000 lbs/day rate = say $500 = $0.25/lb DDT

LABOR AND MATERIAL COSTS SUMMARY

50 lbs/day rate	$0.75 + $5.00 = $5.75/lb DDT
2000 lbs/day rate	$0.75 + $0.25 = $1.00/lb DDT

* The authors wish to thank Mr. Robert E. Cox of Chemical Process Technology Branch, Chemical & Plants Div., Manufacturing Technology Dir of this estimate.

GLOSSARY

DBA: 1,1-dichloro benzilic acid

DDA: 1,1-dichloro-2,2-bis(p-chlorophenyl)acetic acid

DDD: 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane

DDE: 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene

DDT: 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane

DDF: dimethylformamide